Reference: *Biol. Bull.*, **148**: 286–302. (April, 1975)

EFFECTS OF TEMPERATURE ON THE MORPHOLOGY OF HEMOCYTES AND COAGULATION PROCESS IN THE MOLE-CRAB *EMERITA* (=*HIPPA*) *ASIATICA*

M. H. RAVINDRANATH

Department of Zoology, University of Madras, Chepauk, Madras, Tamil Nadu, 600005, India

It can be seen from previous works that the hemocytes in arthropods described by different workers in this field do not lend themselves for a common classification (see literature cited in Ravindranath, 1973, 1974a, 1974b) to enable comparisons of their structure and functions. Previous workers (Jones, 1962; McLaughlin and Allen, 1965) consider that classification based on morphological features of the cells in question may be more valid than any based on their physiological roles. Different functions may be performed by the same cell and apparently different structural types may be performing similar functions. The classification suggested by Jones (1962) for insect hemocytes on the basis of the structural features was found to be applicable to other groups of arthropods (Ravindranath, 1973, 1974a, 1974b). It would be of interest to know whether such a classification can be extended to the hemocytes of decapod crustaceans, in which there is considerable diversity of views (Halliburton, 1885; Hardy, 1892; Cuénot, 1895; Bruntz, 1905; Kollmann, 1908; Tait and Gunn, 1918; George and Nichols, 1948; Toney, 1958; Dall, 1964; Hearing and Vernick, 1967; Wood and Visentin, 1967; Cheney, 1971; and Johnston, Elder and Davies, 1973).

There is also divergence in the views of authors regarding the roles hemocytes play in gelification or coagulation of plasma in arthropods (Grégoire, 1970). A number of authors (Loeb, 1903; Muttkowski, 1924; Yeager, Shull and Farrar, 1932; Yeager and Knight, 1933; and Beard, 1951), believe that gelification of plasma is initiated by a factor resulting from agglutination of hemocytes. Beard (1951) believes that coagulation can be inhibited by keeping all hemocytes in a dispersed state. On the other hand experiments carried out by Grégoire (1953) indicate that agglutination of cells may not play any part in the hemolymph coagulation. The above author, like many other previous investigators (Hardy, 1892; Tait, 1911), considers that the contents of highly unstable explosive corpuscles with hyaline cytoplasm and eccentric, cart-wheel-like nuclei (corpuscles called coagulocytes or explosive corpuscles or cystocytes) play a decisive role in gelification of hemolymph. While this is so, a number of recent investigators, based on light and electron microscopic observations (George and Nichols, 1948; Dumont, Anderson and Winner, 1966; Hearing and Vernick, 1967; Moran, 1971; and Scharrer, 1972) have attributed the function of hemolymph coagulation to granular hemocytes, which differ morphologically from the explosive corpuscles or cystocytes (see Hardy, 1892; Grégoire, 1970). Although direct evidence is lacking to show that dissolved contents of the granules induce coagulation, Scharrer (1972, p. 313) states that "there remains little doubt that the stepwise transformation of the special cytoplasmic inclusions discussed here, culminating in the release of their content into the hemolymph, play a decisive role in clotting process."

In view of the above findings, an attempt has been made in the present study to classify the hemocytes of *Emerita asiatica* before determining their functional role in coagulation. Such a step may obviate the difficulties faced by previous workers who, on account of the terminology adopted by them, were not clear of the identification of the hemocytes involved in one or the other of the functions of the hemocytes.

Materials and Methods

Specimens of the mole-crab *Emerita* (= *Hippa*) *asiatica* were collected at low tides from the shores of Madras Beach, opposite University Campus. Immediately after collection, the specimens were taken to the laboratory and were used for the investigation within six hours. The animals were kept in containers previously filled with sand obtained from the collecting area and were provided with sea water. The size, sex, reproductive and molt cycle stages were recorded prior to collection of blood samples. The stages of the molt cycle were identified using the criteria suggested by Drach (1939).

Blood samples were collected by cutting the first walking leg of the animal. Observations were made on unfixed, fresh preparations as well as on unfixed, stained preparations. The stains used were 0.1% aqueous toluidine blue (BDH 837530) and 0.5% aqueous bromophenol blue. For observations of living cells by phase contrast microscopy, a drop of blood was taken on the glass slide and immediately covered with a cover glass.

Effects of temperature on the morphology of the hemocytes as well as on clotting time were recorded subsequent to immersing the animal in seawater at various temperatures and time following the procedure of Yeager, Shull and Farrar (1932). Initially, the animals were individually kept immersed in sea water for one minute at room temperature. Each animal was taken out, the water was drained and wiped off with filter paper. The region of the leg to be cut was particularly carefully wiped. Care was taken to avoid mixing of sand particles or sea water with the blood sample.

Analyses were carried out in animals immersed for one minute at different temperatures at 5° C intervals from 5 to 45° C. In subsequent experiments the immersion time was prolonged to 2, 5 and 10 minutes. In all these experiments the temperature was maintained in a water bath. Results recorded were based on six experimental animals in each case.

The alterations of hemocytes were recorded after immersion as follows: a stop watch was set at the time of cutting the tip of the leg; the first blood drop was then placed on a clean slide and was covered with a coverslip; the cells were then viewed in phase microscopy under low power, which facilitated viewing the alterations of about 120–200 cells at a time; and the final time was recorded when all the hemocytes in question were degranulated and disintegrated.

The clotting time was determined following the procedure described by Peters and Long (1973). The first drop of hemolymph was placed immediately on a slide and clotting time was determined. The slide was tilted once each 15 seconds, and gelification of plasma was indicated when tilting the slide no longer resulted in conformational change of the drop.

The quick tilt technique of Stewart, Dingle and Odense (1966) was also attempted. In this procedure aggregation and agglutination of hemocytes occurred

readily and the plasma gelification was much delayed when compared to the time obtained with the previous method.

Results

General Observations

The hemolymph of *Emerita asiatica* is a clear pale straw-yellow, watery fluid. Occasionally it is colorless. When a drop of hemolymph is allowed to stand at room temperature (28° C), the fluid transforms into a gel in about three minutes. On long standing, the hemocytic meshes embedded in the gel darken. Gelification of plasma is known to be brought about as a result of agglutination of hemocyte or by alterations of fragile hyaline hemocytes (= coagulocytes, = cystocytes) or by distintegration and degranulation of coarse granular hemocytes. Due to paucity of information regarding the morphology and functions of hemocytes of *Emerita asiatica*, the morphology of hemocytes had to be studied.

Morphology of hemocytes

The hemocytes of *Emerita asiatica* can be divided into six types based on their morphology. Each cell type can be easily distinguished from the other types. The hemocytes were classified using the classification and terminology suggested by Jones (1962). The six types of hemocytes recognized in *Emerita asiatica* are prohemocytes, plasmatocytes, granular hemocytes, cystocytes, spherule cells and adipohemocytes. The general characteristics of the hemocytes are presented in Table I. The hemocyte types are compared with those of other decapods in Table II.

Prohemocytes. These are always small, mostly round to ovoid cells, characterized by small amounts of cytoplasm and the nucleus comprising the greater amount of the cell volume (Figs. 1–4, 11). The size of these cells varies from 7 to 11 μ. These cells could easily be seen in heat fixed preparations of male at intermolt, premolt and freshmolt stages. Occasionally, morphological variations are seen among prohemocytes. In some cases, the cytoplasm may be smooth. In a few cases they can be seen with refractile, boat-shaped granules or with refractile spherules. In some prohemocytes, a big vacuole can be observed giving a signet ring appearance to the cells.

Plasmatocytes. These are rare among circulating hemocytes; they are weakly basophilic cells and exceedingly variable in form (Figs. 5, 6). The cytoplasm contains few non-refractile fine orthochromatic granules. Occasionally they send out blunt pseudopodia. The length of these cells varies from 10 to 18 μ.

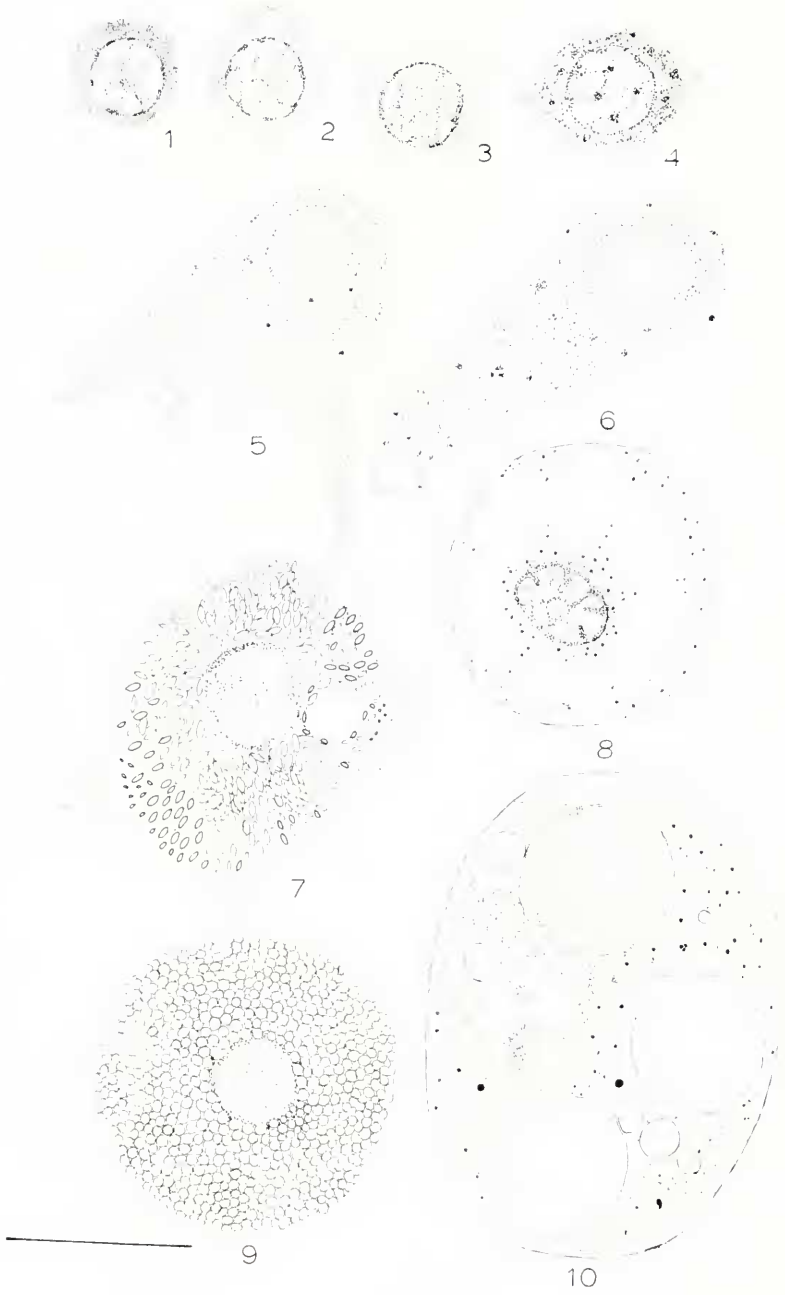
Granular hemocytes. These are basophilic cells, with refractile boat-shaped or elliptical granular inclusions. These cells vary in their length from 12 to 30 μ (Figs. 7, 12). The nucleus is spherical, homogeneous and centrally situated. Its presence is masked by the mass of granules.

The morphology of the granular hemocytes changed significantly when a drop of blood was added to a drop of toluidine blue. The cells swell like balloons, the granules tend to dissolve and become finely granular (Fig. 13). The fine granules accumulated towards the periphery of the cells and exhibited quick jostling move-

TABLE I

The general characters of the hemocyte types of Emerita asiatica.

Characteristics	Prohemocytes	Plasmatocytes	Granular hemocytes	Cystocytes	Spherule cells	Adipohemocytes
Size	7–11 μ	10–18 μ	14–20 μ	15–20 μ	12–22 μ	20–32 μ
Shape	round-oval non-refractile or refractile	oval-spindle polymorphic	oval to polymorphic refractile	oval, non-refractile	round, refractile	oval, refractile
General nature	intensely basophilic, cytoplasm is orthochromatic	basophilic, cytoplasm is orthochromatic in toluidine blue	basophilic, cytoplasma is orthochromatic	weakly basophilic, cytoplasm is orthochromatic	basophilic, cytoplasm is orthochromatic	basophilic, cytoplasma is orthochromatic
Nucleus	homogeneous central	homogeneous central	homogeneous central	heterogeneous, cart-wheel like eccentric	granulated-central	homogeneous-eccentric
Cytoplasmic inclusions	absent or present in various shapes	rare-if present phase-dark, round	boat-shaped, elliptical, refractile	rare, fine granules	spherular	spherular-granular and refractile globular
Behavior	remains same till 1 hr.	changes in shape	degranulates vacuolates and disintegrates, time of alteration varies with temperature	changes soon after exposure	changes very slowly	no change till 1 hr.
In toluidine blue	β	—	(—) or β	α	?	granule: α; spherule; β globule: no color
(In bromophenol blue)						
In bright-field	dark blue	dark blue	green to purple	dark blue	blue	spherule: blue globule: yellow
Under phase	blue	blue	green to purple	blue	purple	spherule: no color globule: yellow



FIGURES 1–10. The appearance of *Emerita* hemocytes under phase contrast. Figures 1–4 show prohemocytes; 2 and 3, vacuolated prohemocytes; 5 and 6, plasmatocytes; 7, a granular hemocyte with characteristic granular inclusions and one vacuole; 8, an ovoid cystocyte with eccentric cart-wheel-like nucleus and vacuolated cytoplasm showing fine granules; 9, a spherule cell with characteristic spherular inclusions; and 10, an adipohemocyte with eccentric nucleus and various sizes of fat-like droplets and inclusions. The scale bar indicates 10 μ.

ments. The cells shrink after some time. The nucleus begins to lose its homogeneity, shape and size (Figs. 14, 15). Occasionally these cells appear to be binucleate.

Cystocytes. These are highly unstable round or oval basophilic cells with eccentric and cart-wheel-like nuclei (Fig. 8). Within a minute of removal of hemolymph, these cells disintegrate and cause glassy veils in the plasma surrounding them. The morphology and the behaviour of cystocytes recall the pattern of coagulation brought about by fragile hyaline hemocytes or coagulocytes of some insects (Grégoire, 1951).

During the alteration of cystocytes, in thin wet films, the nuclei have undergone considerable change. Initially the nucleus of a cystocyte is ellipsoid and homogeneous. Slowly the homogeneity is lost and a fine granular network is seen in the nucleoplasm. After a few minutes, the nucleus becomes round and cart-wheel-like, indicative of nuclear pycnosis and chromatic disaggregation. Associated with the structural modifications, the affinity of the nucleus changes for the reactive groups of the dyes bromophenol blue and toluidine blue. At initial phase, the nucleus stains yellow with bromophenol blue and green with toluidine blue. Finally, it stains blue with the acid dye and purple with the basic dye. The changes in the stainability of the nucleus may signify alterations in the reactive groups of nucleic acids and nuclear proteins.

The orthochromatic cytoplasm of cystocytes contains fine, refractile and β-metachromatic granules, which were in continuous movement. These cells range 15 to 20 μ in size.

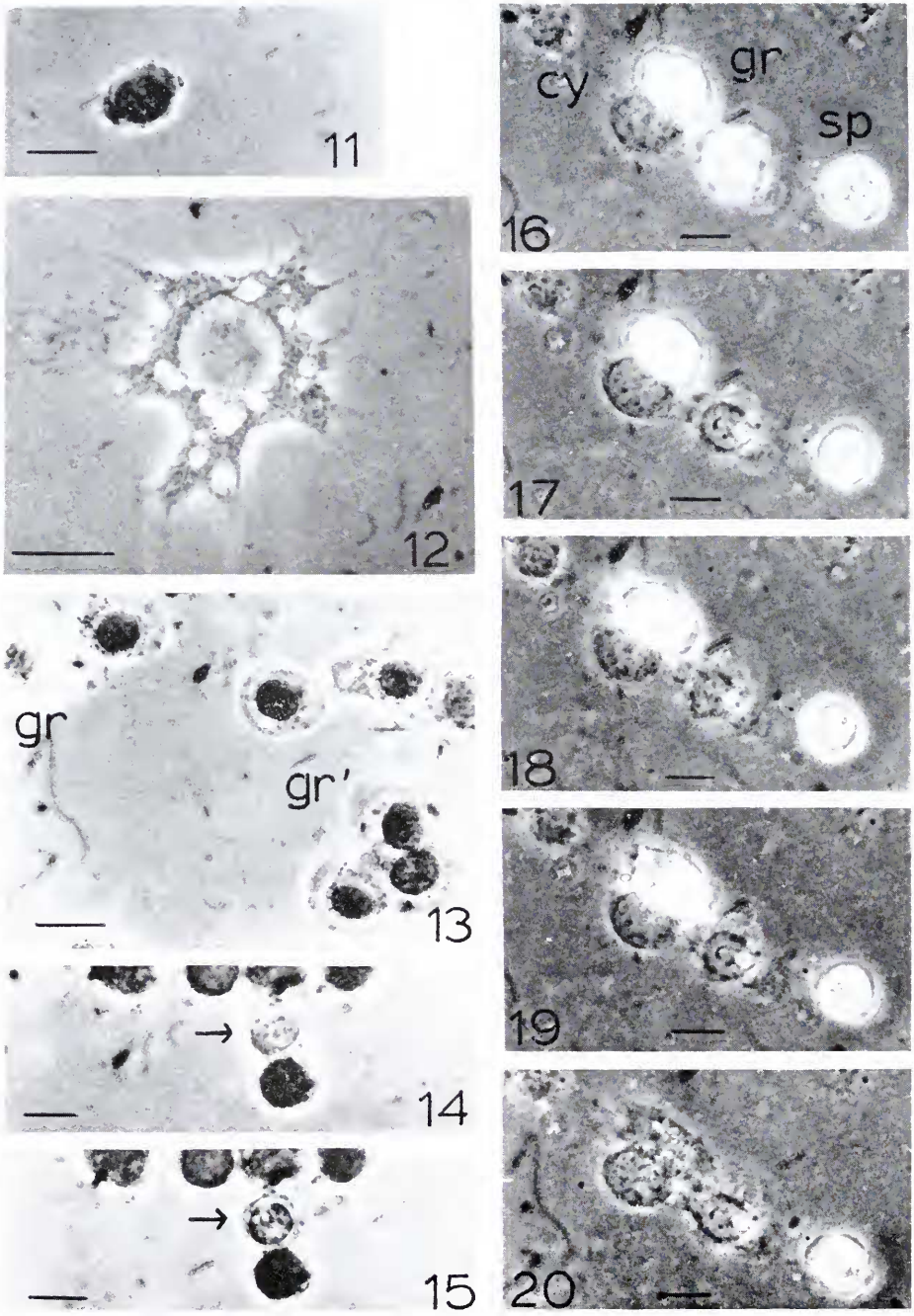
Spherule cells. These are round, refractile basophilic hemocytes with many distinct, uniformly round, acidophilic, refractile spherules (Figs. 9, 16–20). They range in their width from 12 to 22 μ. They do not disintegrate or breakdown into intensely hyaline forms in thin wet films, but after prolonged exposures, some cells become vacuolated and in some, the refractile spherules fuse together (Fig. 20). They do not anastomize to form plasmodia.

The spherules stain dark blue with bromophenol blue. But in phase microscopy, the spherules, soon after adding the dye, show a green color which subsequently become purple. The immobile nature of the spherules and their affinity to aqueous bromophenol blue distinguish this cell type from other hemocytes. This cell type is found in greater numbers only in postmolt period.

Adipohemocytes. On rare occasions, large oval basophilic hemocytes with eccentric nucleus and many refractile droplets or globules of various sizes have been observed in the hemolymph of *Emerita asiatica* (Fig. 10). They have the appearance of small fat-body cells in insects. They measure in length about 20–32 μ.

Observations made on hemocytes of various size groups, sexes, molting and reproductive stages of *Emerita asiatica* reveal that granular hemocytes and cystocytes are the predominant cell types. The percentage of granular hemocytes in fresh preparations varies from 60 to 75%, whereas that of cystocytes varies from 20 to 35%. Other cell types, though rare, are driven into circulation during heat-fixation. No dividing cells were observed among any of the cell types.

When the hemolymph is observed soon after collection, the explosive nature of the cystocytes can be observed. They cause glassy veils in which other cells may get attached. It was noted that the cystocytes are not the only cell type to undergo changes; the granular hemocytes do likewise.



FIGURES 11–20. Photomicrographs of *Emerita* hemocytes. Figure 11 shows a prohemocyte, note the size and cytoplasm : nucleus ratio. The scale bar indicates 10 μ. Figure 12 shows a hemocyte after prechilling, note the well-spread granular hemocyte with ellipsoid granules and

Events associated with coagulation

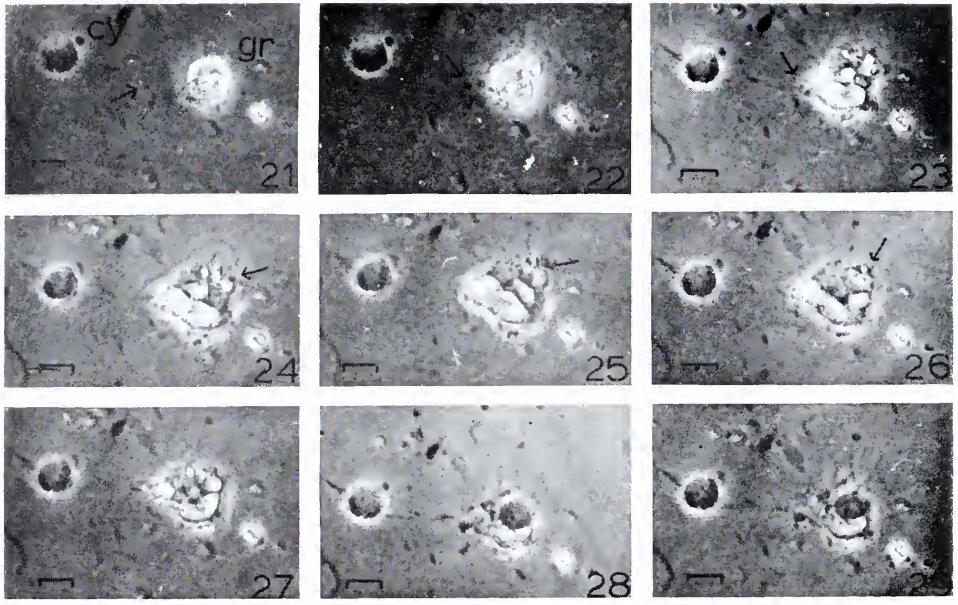
Alterations in granular hemocytes. Figures 13–15, 16–20 and 21–29 show the alterations undergone by granular hemocytes in thin wet films and in stained preparations. Individually these cells exhibit "atypical amoeboid motion" similar to that exhibited by the granular hemocytes of an insect *Blaberus giganteus* (Arnold, 1959, 1961). This process involves the separation of the cell's cytoplasm into an outer hyaline ectoplasm and an inner refractile granular endoplasm. Movement is initially accomplished by the flow of cytoplasm in the region of ectoplasm. The flow of cytoplasm thus results in formation of blunt pseudopodia with scalloped edges. The granular endoplasm did not stream or enter the pseudopodia but some turbulence of tension seemed to occur there. It merely followed the advancing hyaline ectoplasm and conformed to its outlines. These outlines became modified. The base broadened with the inflow of endoplasm, the pseudopodium became lamellar, and subsequently changed completely to the typical amorphous streaming pseudopodium. Under these conditions, vacuoles appeared in the center of granular endoplasm and the boat-shaped granules began to lose their refractility (Figs. 21–23). Figure 12 reveals a granular hemocyte at this stage with numerous pseudopodia and refractile vacuoles. As they lose their refractility, the edges of these inclusions are sharp black and the enclosed granular space bright.

The slow cytoplasmic movements in the granular hemocytes are associated with haphazard jostling of the granules within the small areas of the cells. The surface area of the cell begins to expand to thrice the original cell size. Few granules that have reached the ectoplasm, continually move in and out of it in a shuttling motion. In the ectoplasm, the granules circled irregularly as individuals or as small chains that percolated in and out through an apparent meshwork of relatively stationary granules, which themselves occasionally joined in the action. The shuttling granules slowly dissolved in the ectoplasm and disappeared. The enlarging vacuoles pushed the endoplasmic granules to the periphery of the cells, where they dissolved quickly (Figs. 24–27). The contents of the vacuoles were liberated into the plasma (Figs. 27–29), finally leaving the cytoplasmic meshwork (Fig. 29). This facilitated the interlinking of hemocytes and the agglutination of cells of the same class.

A feature of interest in the alteration of granular hemocyte is that it is initiated quickly in cells which are in the vicinity of bursting cystocytes (Figs. 16–20). The elastic fiber (resulting from bursting of a cystocyte) along with a phase dark granule liberated from another cystocyte were observed to reach a granular hemocyte (Figs. 21–23) prior to the commencement of alterations in the latter.

---

vacuoles, and the fine pseudopodial projections. While the granules have lost their refractility, the vacuoles retain theirs. The scale bar indicates 10 μ. Figure 13 shows granular hemocytes in toluidine blue. The granules, while refractile, do not take up the stain (gr); note the balloon-shaped appearance of the cell and the dissolving granules (gr'). The scale bar indicates 10 μ. In Figures 14 and 15, note the changing shape and staining affinity of the nucleus of the shrunken granular hemocyte. The scale bars indicate 10 μ. Figures 16 through 20 show the alterations of granular hemocytes in the vicinity of burst cystocytes (after immersion of the animal in sea water at 35° C for five minutes). The time interval between each exposure is one minute. Note: cy, cystocytes; gr, granular hemocyte; and sp, spherule cells. The change occurred in the spherule cell in Figure 20. The scale bars indicate 10 μ.



FIGURES 21–29. Photomicrographs of the stepwise alterations in the granular hemocytes (after immersion of the animal in the sea water at 10° C for one minute). The time interval between each exposure is two minutes. Note: cy, cystocyte; and gr, granular hemocyte. The different figures show vacuolization, loss of refractility of granules, dissolution of granules, enlargement and fusion of vacuoles, liberation of vacuolar contents into plasma, and disintegration and anastomosis of cytoplasm. Note a cytoplasmic fiber arising from the cystocyte connecting to the granular hemocyte. The arrows in Figures 21, 22, and 23 show the movement of a phase dark granule in the fiber. The granule from the cystocyte moves and reaches the granular hemocyte. The arrows in Figures 24, 25, and 26 show the jostling granules after losing their refractility. Note their dissolution in the cytoplasm, from which they do not emerge. The scale bars indicate 10 μ.

Similar alterations in the granular hemocytes were also observed in conglomerates of the hemocytes. While complete alterations were observed in the cells found in the periphery, the cells inside the clusters showed different stages of incompleteness. In some cells, a sudden stoppage of the movement of granules was observed while, in some others, the granules remained refractile for more than an hour. Similarly, Grégoire (1970, Fig. 14) observed no distintegration or degranulation in the clusters of granular hemocytes of *Limulus polyphemus*. The sudden stoppage in the movement of granules calls to the mind the observations of Harvey (1942) on the movement of granules during cyclosis in the cells of *Nitella*. He observed that any sudden decrease or increase in temperature caused a shock stoppage of the movement of granules.

Effect of temperature on alterations of granular hemocytes. To determine whether the alterations in the granular hemocytes lead to coagulation of the hemolymph, attempts were made to investigate whether the factors known to influence the coagulation also affect the alterations in the hemocytes. One such factor that is known to influence clotting time is temperature (Dean and Vernberg, 1966).

TABLE II

The classification and terminology of hemocytes of Emerita asiatica *compared with those of other decapods.*

Present study	Hardy, 1892	Cuenot, 1895	Kollmann, 1908	Tait and Gunn, 1918	George and Nichols, 1948	Toney, 1958	Dall, 1964	Wood and Visentin, 1967
Emerita asiatica	*Astacus fluviatilis*	*Astacus fluviatilis*	List A	*Astacus fluviatilis*	List B	*Cambarus sp; Callinectus sapidus.*	*Metapanaeus mastersii*	*Orconectus virilis*
Prohemocyte	—	amibocyte I	hyaline leucocyte I	—	lymphoid cell (Type-I)	lymphoid cell or monocyte	lymphocyte	hyaline cell I
Plasmatocyte	—	amibocyte II	hyaline leucocyte II	thigmocyte	colorless, semihyaline thigmotoctic amoebocyte (Type-II)	—	thigmocyte	hyaline cell II
Granular hemocyte	eosinophil corpuscle	amibocyte à granulations acidophiles (III)	leucocyte granules Pl.II (21,22)	eosinophil, granular amoebocyte	cells with coarse refractile acidophilic granule (Type-III & IV)	explosive refractive granulocyte	large granular amoebocyte	granulocyte.
Cystocyte	explosive corpuscle	amibocyte-II (degenerative)	leucocyte (degenerative) Pl.II(ii)	explosive corpuscle	—	explosive refractive granulocyte	—	—
Spherule cell	basophil	cellules proteiques	cellules spherules	—	Type III of *Cambarus bastoni*	—	—	—
Adipohemocyte	—	—	—	—	—	—	—	—

List A: *Astacus fluviatilis, Palemonetes varians, Palemon serratus, Gebia deltura, Maia squinado, Pisa tetraodon, Carcinus maenas, Portunus depurator, Portunus puber, Corystes cassivelaunus, Pagurus bernhardus, Dromia vulgaris, Galathea intermedia, Galathea squamifera.*
List B: *Libinia dubia, Uca pugnax, Hippa talpoida. Panopius herbstii, Callinectes sapidus, Cambarus bastoni.*

The effect of temperature on the alterations of granular hemocytes is shown in the graph (Fig. 30). The time interval between exposure of blood and complete disintegration of granular hemocytes is recorded after immersion in a seawater bath at the various temperatures and intervals referred to earlier. The cells are fixed after one-minute immersion at 45° C. Lowering of the temperature from 15° C to 5° C results in progressive delay of the alterations of granular hemocytes. On the other hand, the rate of alterations was quickened progressively with increasing temperature. The trend in the temperature-dependent alterations of granular hemocytes remained more or less the same irrespective of the time interval of immersion. But, the time taken for alteration at a particular temperature decreased with increasing immersion time up to five minutes. The delay in the alteration of granular hemocytes at a particular temperature after one-minute immersion

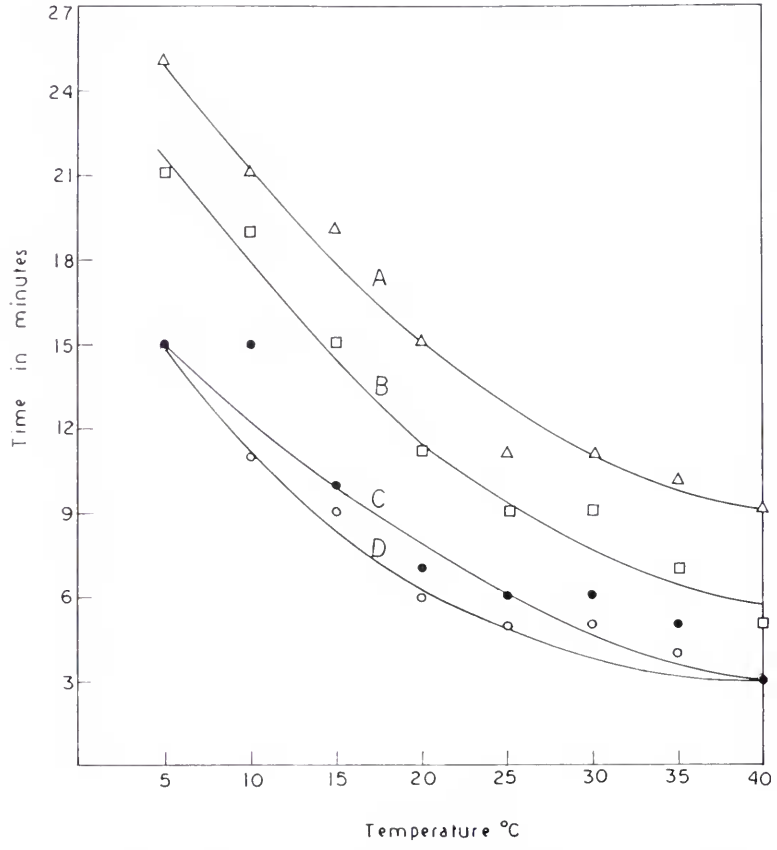


FIGURE 30. Shows the time taken for alteration of the granular hemocyte at different temperatures; observations were made on blood samples collected after immersing the animal in sea water at different time intervals. Triangles (A) indicate immersions of one minute; squares (B), of two minutes; dark circles (C), of five minutes; open circles (D), of ten minutes.

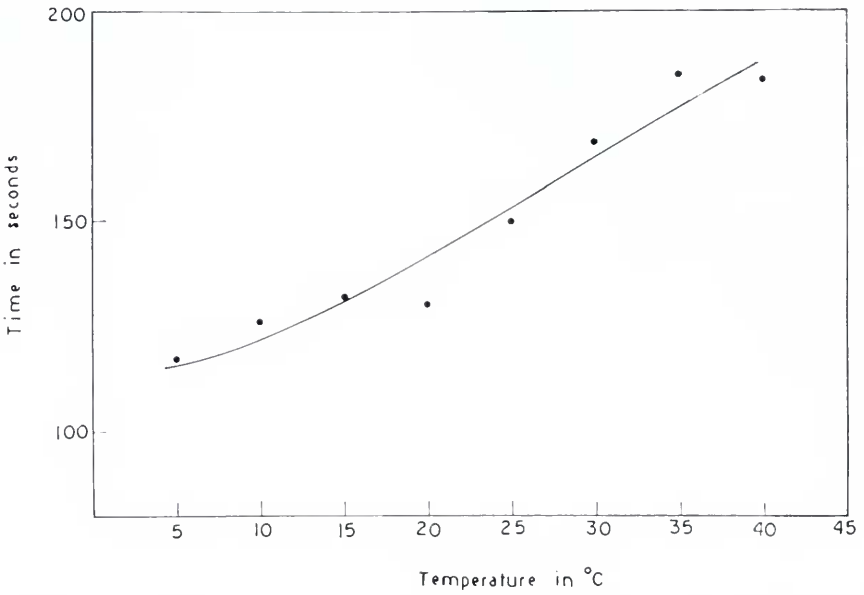


FIGURE 31. Shows the time taken for gelification of plasma at different temperatures. Observations were made on blood samples collected after immersing the animal in sea water for five minutes.

probably reflects the shock response of the animal to sudden change in the temperature of the environment. The animal appears to recover from the shock within five to ten minutes, for there is no significant difference in the time taken for the alteration at any temperature after five- and ten-minute immersions.

On the contrary, the clotting times recorded at different temperatures show an opposite trend (Fig. 31). It was noted that the times taken for gelification of plasma increase with temperature, and here the agglutination of hemocytes did not precede plasma gelification.

DISCUSSION

The observations reported in the present study on the hemocytes of *Emerita asiatica* solve a long standing confusion in the nomenclature of decapod crustaceans (Table II) and enable comparison of different types of hemocytes of crustaceans with the hemocyte types of other arthropods. Absence of mitotically dividing cells in the plasma, occurrence of differences among prohemocytes and the wide size range of different major hemocyte types support the view put forward earlier (Ravindranath, 1973, 1974a, 1974b, 1974c) that prohemocytes may be the progenitors of the various major cell classes.

One of the striking features in the composition of the hemocytes of *Emerita asiatica* is the predominance of one cell type, the granular hemocyte. Such a feature is not uncommon among decapods and king-crabs (see Schulz 1925; George

and Nichols, 1948; Dumont, Anderson and Winner, 1966). An interesting aspect of the granular hemocyte is the nature of granular inclusions, which are refractile and boat-shaped. Such inclusions are also present in the granular hemocytes in insects, *Carausius morosus* (Millara, 1947, Pl.III Figs. 2 and 3), *Locusta migratoria* (Hoffmann, 1967), *Blaberus discoidalis, B. giganteus* and *Leucophaea maderae* (Arnold, 1972, Figs. 105 and 123); and also in *Peripatus sp.* (Grégoire, 1955, Figs. 5 and 6); in chilopods, *Lithobius forficatus* (Grégoire, 1970); arachnids, *Limulus polyphemus* (Grégoire, 1955, Figs. 7 and 14; Dumont, Anderson and Winner, 1966); and in several species of scorpions (Kollmann, 1908; Ravindranath, 1974a). But such a cell type does not fall into any one of the categories of hemocytes classified by Jones (1962).

Another feature of interest is that the prohemocytes, plasmatocytes, spherule cells and adipohemocytes are rare in fresh preparations but common in heat-fixed preparations. Jones (1962) has suggested that this method of preparation drives the hemocytes into circulation from the sites of their accumulation. It is also possible that this method may alter the morphology of cell types.

It is known that the clotting or coagulation of the blood of arthropods consists of two physiologically distinct processes which can occur independently or together: initially hemocyte agglutination or cell coagulation occurs and this may be followed by gelification of the plasma, termed plasma coagulation (Grégoire and Tagnon, 1962). In *Emerita asiatica*, the hemocyte coagulation and plasma gelification occur independently. The hemocytes remain in a dispersed state when plasma gelification has occurred. This finding derives support from the observations of Grégoire (1953). In insects, he suggested that gelification of plasma is initiated by cystocytes. The observations made here and by Jones (1962) support this view. They reveal that cystocytes undergo changes within a minute or two of exposure, irrespective of the temperature differences. Similar findings were reported in the works of Hardy (1892) and Grégoire and Tagnon (1962).

The plasma gelifies swiftly at colder temperatures, a finding which closely parallels the reports of Peters and Long (1973) but which is in contradiction to the results of Dean and Vernberg (1966) and Joshua, Fischl, Henig, Ishay and Gitter (1973). This trend in gelification in relation to different temperatures is quite opposite to that of the alterations of granular hemocytes.

These findings are in accordance with those of Hardy (1892, p. 170), who observed in *Astacus* that "the eosinophil cells (or granular hemocytes) remained unchanged and alive for a considerable time after the blood has clotted." In the same animal, Tait and Gunn (1918) have also reported that no coagulation follows the cytolysis of the eosinophil amoebocytes (= granular hemocytes) which occurs after cytolysis of explosive corpuscles (=cystocytes). Grégoire (1970) likewise reported that gelification occurred when all the hemocytes (except cystocytes) had still retained most of their granules intact. All these observations cast doubt on the direct role of granular hemocytes in coagulation.

The stepwise alterations of the granular hemocytes lead to cellular agglutination or plasmodial formation. This kind of cellular agglutination is an independent process in *E. asiatica* and does not lead to plasma gelification, but takes place subsequent to it. Hemocyte agglutination was reported in other crustaceans (see Table I of Grégoire and Tagnon, 1962) and also in *Limulus polyphemus* (Loeb, 1903;

Copley, 1947; Kenney, Belamarich and Shepro, 1972). All these authors have shown that low temperatures retard the aggregation and agglutination of *Limulus* granular hemocytes. Kenney *et al.* (1972) have suggested that the aggregation-promoting factor is heat-labile. Similarly the temperature-dependent alterations of granular hemocytes of *Emerita asiatica* suggest that the factor promoting the alterations of the granular hemocytes may also be heat-labile. Further, the observations made in the present study (Figs. 21–22) indicate that the factor in question may be liberated from cystocytes. In this regard the observation, that the first signs of alteration of granular hemocytes occur only in the vicinity of cystocytes, is of considerable significance.

It appears in the light of the present investigation that cystocytes may perform two functions: (1) they promote plasma gelification, and (2) they initiate the stepwise transformation of granular hemocytes, which in turn leads to agglutination of cells and to the formation of a meshed network. The significance of the dissolution of the granules is not clear at present.

Another interesting feature observed in the present study is the similarity in the changing pattern of nuclei of both cystocytes and granular hemocytes. A strikingly parallel feature was reported among the explosive corpuscles (= cystocytes) and eosinophil corpuscles (= granular hemocytes) of *Astacus* by Hardy (1892, p. 169), who observed that "the nucleus (of eosinophil corpuscle) comes into view and acquires that intense distinctness which we noticed as such a remarkable features of the rigor mortis, or clotting, of the nuclei of the explosive corpuscles." Dumont, Anderson and Winner (1966) traced the changes that take place in the nucleus ultrastructurally. Although the significance of the changes that occur in the nucleus of these cell types is not clear, the similarity in the behaviour of the nuclei in both these cell types suggests an ontogenic relationship between them.

I am greatly indebted to Professors Dr. G. Krishnan and Dr. K. Ramalingam and to Drs. M. H. Rajeswari-Ravindranath and T. Subramoniam for reading the manuscript and making many valuable suggestions for its improvement. Sincere appreciation is expressed to Dr. S. Raghukumar for taking practically all the photographs upon which the supporting observations were based. I am also thankful to Professor Dr. C. V. Subramaniam, Professor of Botany, for extending necessary facilities.

Summary

1. The hemocytes of *Emerita asiatica* have been studied in fresh preparations by phase contrast microscopy and also after staining.

2. With phase contrast microscopy, the following categories of hemocytes can be identified: (a) nondividing prohemocytes, (b) pleomorphic plasmatocytes, (c) intact and altering granular hemocytes, (d) quickly lysing cystocytes, (e) intact spherule cells, and (f) adipohemocytes.

3. This classification and terminology solve a long standing confusion in the nomenclature of hemocytes of decapods and enable comparisons between the different hemocyte types of crustaceans and the hemocyte types of other arthropods to be made.

4. Cystocytes and granular hemocytes constitute more than 95% of the hemocytes.

5. In thin wet films, the granular hemocytes undergo alterations which include loss of shape, refractility and granules, followed by vacuolization and disintegration leading to agglutination of the cells.

6. The alterations of the granular hemocytes are temperature-dependent, and lowering of the temperature results in progressive delay of the alterations.

7. There is no correlation between alteration of granular hemocytes and clotting time at different temperatures. At lower temperatures, gelification of plasma occurs when the granular hemocytes remain unaltered. The observations reveal that the granular hemocytes may not play any role in gelification of plasma.

8. Further, the process of cellular agglutination does not play any part in the phenomenon of plasma gelification, unlike this process in insects as reported by Grégoire.

9. Cystocytes, which have been referred to as explosive corpuscles by previous crustacean workers, disintegrate within a minute or two of exposure at all temperatures. Evidence supports their role in plasma gelification.

10. The observations also indicate that the substances liberated from the cystocytes initiate the stepwise transformation of granular hemocytes.

LITERATURE CITED

Arnold, J. W., 1959. Observations on amoeboid motion of living haemocytes in the wing veins of *Blaberus giganteus* (L) (Orthoptera: Blattidae). *Can. J. Zool.*, **37**: 371–375.

Arnold, J. W., 1961. Further observations on amoeboid haemocytes in *Blaberus giganteus* (L) (Orthoptera: Blattidae). *Can. J. Zool.*, **39**: 755–766.

Arnold, J. W., 1972. A comparative study of the haemocytes (blood cells) of cockroaches (Insecta: Dictyoptera: Blattaria), with a view of their significance in taxonomy. *Canad. Ent.*, **104**: 309–348.

Beard, R. L., 1951. Experimental observations on coagulation of insect hemolymph. *Physiol. Zool.*, **23**: 47–57.

Bruntz, L., 1905. Etudes physiologiques sur les phyllopodes branchipiodes. Phagocytose et excretion. *Arch. Zool. Exp. Gen.*, **76**: 1–67.

Cheney, D. P., 1971. A summary of invertebrate leucocyte morphology with emphasis on blood elements of the Manila Clam, *Tapes semidecussata*. *Biol. Bull.*, **140**: 353–368.

Copley, A. F. L., 1947. The clotting of *Limulus* blood. *Fed. Proc.*, **6**: 90–91.

Cuenot, L., 1895. Etudes physiologiques sur les crustacees decapodes. *Arch. Biol. Liege.*, **13**: 245–303.

Dall, W., 1964. Studies on the physiology of shrimp, *Metapenaeus mastersii* (Haswell) (Crustacea: Decapoda: Penaeidae). I. Blood Constituents. *Aust. J. Mar. Freshw. Res.*, **15**: 145–61.

Dean, J. M., and F. J. Vernberg, 1966. Hypothermia and blood of crabs. *Comp. Biochem. Physiol.*, **17**: 19–22.

Drach, P., 1939. Mue et cycle d'intermue chez les crustaces decapodes. *Ann. Inst. Oceanogr.*, **19**: 103–391.

Dumont, J. N., E. Anderson, and G. Winner, 1966. Some cytologic characteristics of the hemocytes of *Limulus* during clotting. *J. Morphol.*, **119**: 181–217.

George, W. G., and J. Nichols, 1948. A study of the blood of some crustacea. *J. Morphol.*, **83**: 425–443.

Grégoire, C., 1951. Blood coagulation in arthropods. II. Phase contrast microscopic observations on hemolymph coagulation in sixty-one species of insects. *Blood*, **6**: 1173–1198.

Grégoire, C., 1953. Blood coagulation in arthropods. III. Reactions of insect hemolymph to coagulation inhibitors of vertebrate blood. *Biol. Bull.*, **104**: 372–393.

Grégoire, C., 1955. Blood coagulation in arthropods. VI. A study by phase contrast microscopy of blood reactions in vitro in Onychopora and in various groups of arthropods. *Arch. Biol. Paris*, **66**: 489–508.

Grégoire, C., 1970. Haemolymph coagulation in arthropods. *Symp. Zool. Soc. London.*, **27**: 45–74.

Grégoire, C., and H. J. Tagnon, 1962. Blood coagulation. Pages 435–482 in M. Florkin and H. S. Mason, Eds., *Comparative Biochemistry*, Volume 4. Academic Press, New York and London.

Halliburton, W. E., 1885. On the blood of the decapod crustacea. *J. Physiol.*, **6**: 300–335.

Hardy, W. B., 1892. The blood corpuscles of the crustacea, together with a suggestion as to the origin of the crustacean fibrin-ferment. *J. Physiol.*, **13**: 165–190.

Harvey, E. N., 1942. Hydrostatic pressure and temperature in relation to stimulation and cyclosis in *Nitella flexilis*. *J. Gen. Physiol.*, **25**: 855–863.

Hearing, V., and S. H. Vernick, 1967. Fine structure of the blood cells of the lobster *Homarus americanus*. *Chesapeake Sci.*, **8**: 170–186.

Hoffmann, J. A., 1967. Etude des hemocytes de *Locusta migratoria* L. (Orthoptere). *Arch. Zool. Exp. Gen.*, **108**: 251–291.

Johnston, M. A., H. Y. Elder, and P. S. Davies, 1973. Cytology of *Carcinus* haemocytes and their function in carbohydrate metabolism. *Comp. Biochem. Physiol.*, **46A**: 569–582.

Jones, J. C., 1962. Current concepts concerning insect hemocytes. *Amer. Zool.*, **2**: 209–246.

Joshua, H. J., F. B. Fischl, E. Hening, J. Ishay, and S. Gitter, 1973. Cytological biochemical and bacteriological data of the hemolymph of *Vespa orientalis*. *Comp. Biochem. Physiol.*, **45B**: 167–175.

Kenny, D. M., F. A. Belamarich, and D. Shepro, 1972. Aggregation of horseshoe crab (*Limulus polyphemus*) amoebocytes and reversible inhibition of aggregation by EDTA. *Biol. Bull.*, **143**: 548–567.

Kollmann, M., 1908. Recherches sur les leucocytes et le tissue lympoide des invertebres. *Ann. Sci. Natur. Zool.*, **8**: 1–238.

Loeb, L., 1903. On the coagulation of the blood of some arthropods and on the influence of pressure and traction of the protoplasm of the blood cells of arthropods. *Biol. Bull.*, **3**: 301–318.

McLaughlin, R. E., and G. Allen, 1965. Description of hemocytes and the coagulation process in the Boll Weevil, *Anthonomus grandis* Boheman (curculionidae). *Biol. Bull.*, **128**: 112–124.

Millara, P., 1947. Contribution a l'etude cytologique et physiologique des leucocytes d'insects. *Bull. Biol. France and Belgium*, **81**: 129–153.

Muttkowski, R. A., 1924. Studies on the blood of insects. III. The coagulation and clotting of insect blood. *Bull. Brooklyn Entom. Soc.*, **19**: 128–144.

Moran, D. T., 1971. The fine structure of cockroach blood cells. *Tissue Cell*, **3**: 413–422.

Peters, R. I., and G. E. Long, 1973. Some alterations in hemolymph proteins of *Hemigrapsus nudus* associated with temperature acclimation. *Comp. Biochem. Physiol.*, **46A**: 207–213.

Ravindranath, M. H., 1973. The hemocytes of a millipede, *Thyropygus poscidon*. *J. Morphol.*, **141**: 257–268.

Ravindranath, M. H., 1974a. The hemocytes of a scorpion, *Palamneus swammerdami*. *J. Morphol.*, **144**: 1–10.

Ravindranath, M. H., 1974b. The hemocytes of an isopod, *Ligia exotica* Roux. *J. Morphol.*, **144**: 11–22.

Ravindranath, M. H., 1974c. Changes in the population of circulating haemocytes during moultcycle phases of the millipede, *Thyropygus poscidon*. *Physiol. Zool.*, in press.

Scharrer, B., 1972. Cytophysiological features of hemocytes in cockroachs. *Z. Zellforsch.*, **129**: 301–319.

Schulz, N. F., 1925. Crustacean. Pages 669–746 in H. Winterstein, Ed., Handbuch der vergleichenden physiologie, **1**(1): Physiologie der Korperslifte. Gustav Fischer, Jena.

Stewart, J. E., J. R. Dingle, and P. H. Odense, 1966. Constituents of the hemolymph of the lobster *Homarus americanus* Milne Edwards. *Can. J. Biochem.*, **44**: 1447–1459.

Tait, J., 1911. Types of crustacean blood coagulation. *J. Mar. Biol. Ass. U.K.* **9**: 191–198.

Tait, J., and J. D. Gunn, 1918. The blood of *Astacus fluviatilis:* a study in crustacean blood with special reference to coagulation and phagocytosis. *Quart. J. Exp. Physiol.*, **12**: 5–80.

Toney, M. E., Jr., 1958. Morphology of the blood cells of some crustacea. *Growth,* **22**: 35–50.

Wood, P. J., and L. P. Visentin, 1967. Histological and histochemical observations of the hemolymph cells in the crayfish, *Orconectes virilis*. *J. Morphol.,* **123**: 559–568.

Yeager, J. F., and H. H. Knight, 1933. Microscopic observations on blood coagulation in several species of insects. *Ann. Entomol. Soc. Amer.,* **26**: 591–602.

Yeager, J. F., W. E. Shull, and M. D. Farrar, 1932. On the coagulation of blood from the cockroach, *Periplaneta orientalis* (Linn.) with special reference to blood smears. *Iowa State Coll. J. Sci.,* **6**: 325–346.